A Sensitive Method for Identification of Porcine Contaminant in Unprocessed Food by PCR Amplification Technique

Metode Sensitif untuk Identifikasi Pencemaran Babi pada Makanan Tanpa Diolah dengan Teknik Amplifikasi PCR

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Abstract

In the era of globalization currently, it is almost impossible to avoid from either processed or non-processed food from overseas. This research was aimed to examine the sensitivity of PCR technique in the identification of small contents (%) of porcine contaminant in the fresh beef meat. Five-tiny porcine contents of 0.05; 0.10; 0.15; 0.20 and 0.25% in a total weight of 5 gram mixed meat were examined in this study. Positive control (100% porcine meat) and negative control (100% beef meat) were also involved. Five mixed meat, positive and negative control were collected their DNA by using commercial kit (QIAGEN). A pair of specific primer for porcine Leptin was used for DNA amplification. PCR optimization was conducted in prior to PCR work to get approximately annealing temperature of the Leptin primer. Two PCR cycles of 25 and 35 were applied in the PCR works. PCR products were visualized in the 10–20% PAGE Gradient. Results showed that Lepten fragments were identified at all 5 mixed meat samples and positive control (100% pork) with size of 152bp, however it was none Leptin in the negative control (100& beef). Amplification of DNA with 35 cycles of PCR showed more clear band compared to that 25 cycles of PCR in all samples. This study suggests that a quick method of PCR amplification with 35 cycles is suitable as a sensitive method for identification of up to 0.05% porcine contaminant in fresh beef meat.

Key words: Leptin, sensitivity, PCR method, Contamination, porcine

Abstrak

Pada era globalisasi akhir-akhir ini, tidak mungkin menghindar dari masuknya bahan makanan olahan atau tanpa diolah dari luar negeri. Tujuan penelitian ini yaitu menguji sensitivitas (kerentanan) teknik PCR untuk deteksi kandungan rendah kontaminan daging babi pada daging sapi mentah. Sebanyak 5 tingkat kontaminan daging babi (0,05; 0,10; 0,15; 0,20 dan 0,25%) dalam 5 gram berat total campuran daging telah diuji. Ke lima campuran daging dan 100% daging sapi serta 100% daging babi, dikoleksi DNA nya menggunakan kit DNA (QIAGEN). Satu pasang primer spesifik untuk *porcine Leptin* digunakan dalam amplifikasi DNA dengan kit PCR. Suhu annealing ditentukan dengan optimasi PCR terlebih dahulu. Dua siklus PCR (25 dan 35) diaplikasikan dalam amplifikasi. Produk PCR divisualisasi pada 10–20% gradient PAGE untuk spesifik *porcine Leptin.* Hasil menunjukkan bahwa ukuran potongan Leptin (152pb) telah teridentifikasi pada ke lima sampel sampuran maupun pada control positif (*pork*), namun tidak terindikasi pada kontrol negatif (daging sapi). PCR dengan 35 siklus menghasilkan tampilan pita lebih baik dari 25 siklus. Studi ini menyarankan bahwa PCR dengan 35 siklus dapat digunakan sebagai metode cepat untuk identifikasi pencepamaran daging babi dengan dengan tingkat sensitivitas pencemaran daging babi sampai 0,05%.

Kata kunci: Leptin, sensitiviitas, PCR method, pencemaran, daging babi